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The Unsaturated Acids of Salmon Oil

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BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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DISCUSSION OF PROBLEM

Unsaturated aliphatic acids are an almost universal constitutent of fats and fatty oils. Upon the degree of unsaturation, more than upon any other one characteristic, depends the chemical and physical properties of the fat. Saturated fats are solid, with melting points at body temperatures or higher; unsaturated fats are soft, pasty masses or liquids at ordinary temperatures. Saturated fats are stable in the presence of oxygen or at most become rancid due to various hydrolytic cleavages or oxidation of the glycerol molecule; unsaturated fats absorb oxygen, showing marked increase in weight, and as a result of this absorption of oxygen may even be converted to hard, resin-like masses, a property made use of in paints and varnishes.

Unsaturated fats occur in both the animal and vegetable kingdoms.

Oleic acid, ($CH_3\ (CH_2)_7\ CH{=}CH\ (CH_2)_7\ COOH$) and

Linolic acid, ($CH_3\ (CH_2)_4\ CH{=}CH_2\ CH{=}CH\ (CH_2)_7\ COOH$

are found in both animal and vegetable fats.

Linolenic acid, $C_2H_5\ CH{=}CH\ CH_2\ CH{=}CH\ CH_2\ CH{=}CH\ (CH_2)_7\ COOH$

is found only[1] (?) in vegetable fats, Clupanodonic acid, ($C_{18}\ H_{28}\ O_2$), structure yet unknown, and other tetraunsaturated acids are supposed to occur in animal fats and fat-like bodies only, as in fish oils and nervous tissue.

The unsaturated acids and their glycerides in addition to absorbing oxygen may add halogens directly to the double bonds resulting in the formation of saturated derivatives. The bromine addition products formed with the hexa- and octo- unsaturated acids and their glycerides are very insoluble in ether and their formation has been used as a basis for the quantitative study of the composition of these fats, and for the detection and determination of these fats in mixtures, Since the derivatives were first prepared from vegetable oils, it has become customary to refer to all such derivatives as "hexabromides" regardless of their

source.

An article by Bailey and Johnson[2] which has recently appeared, discussing the hexabromides of Salmon oil, has called our attention to this custom, and the analyses given in this paper have been made with the purpose of proving the accuracy or inaccuracy of such nomenclature.

The plan of attack has been in brief: (1) The preparation of the free fatty acids of Salmon oil, (2) The preparation of the ether insoluble bromides from the mixed fatty acids, (3) The determination of the bromine content of the brominated acids.

HISTORICAL

Our present knowledge of the composition and constitution of the fluid fatty acids occurring in natural fluid glycerides dates no further back than the year 1888, when K. Hazura[3], together with a number of collaborators, published a series of exceedingly important papers. Up to that time the fluid portion of oils, drying and non-drying, was described as olein. Hazura concluded from his investigations that the unsaturated fatty acids from fixed oils take up as many hydroxyl groups as there are free valences, yielding saturated hydroxy-fatty acids containing the same number of carbon atoms as the original molecule. He also showed that the unsaturated fatty acids in question combined directly with bromine, as many bromine atoms being fixed as there are free valences. He thus differentiated between acids of different degrees of non-saturation. Hehner and Mitchell[4] afterwards worked out a quantitative method for the determination of the hexabromide value. Their method depends on the differences in solubility of the brominated glycerides in ether, and of the brominated fatty acids in acetic acid, those compounds with higher percentage of bromine being much more insoluble. Various oils were used - linseed, rape, codliver, and shark.

Proctor and Bennett[5] used a different solvent-carbon tetrochloride instead of ether-and precipitated the bromides with absolute alcohol. L.M.Tolman[6] used Hazura's methods for separation and estimation of the bromine compounds but changed the mechanical details and thus found the use of a different solvent, as done by Proctor and Bennett, unnecessary.

The method employed in precipitating the bromides was to dissolve the oil or fatty acid in absolute ether, acidify with acetic acid, cool in a bath of ice water and then place it in a centrifuge and whirl. The bromides thus collected are filtered, washed, dried, and weighed. The determination of bromine was effected by combustion with sodium peroxide in the bomb of a Parr calorimeter. The amounts of fatty acids were calculated from the weight of the precipitate and the percentage of bromine found in them.

Sutcliffe[7], as a result of his investigations, recommended that after bromination the mixture stand over night at 11°C. before filtering and washing the precipitate, whereas Tolman allowed the mixture to stand only thirty minutes after bromination. He found that it was also necessary to add enough bromine to give a good red color instead of merely a yellowish brown as recommended by previous investigators. Gemmell[8] later questioned Sutcliffe's method but Sutcliffe replied by demonstrating that if his directions were carefully followed his method could be used satisfactorily.

H.S.Bailey and J.M.Johnson used a proceedure which is a combination of the methods of Tolman and Sutcliffe, and is as follows:

"About one gram of oil is weighed into a tared weighing tube one inch in diameter and six inches long, 25cc. of absolute ether are added, and the mixture cooled in ice water. Next there is added very slowly drop by drop from a small burette a mixture composed of 5cc. of bromine and 25cc. of glacial acetic acid. This reagent makes an excellent brominating agent and allows the bromine to be added more uniformly and gradually than when pure bromine is used. Besides, it

gives the acetic acid necessary for a proper precipitation of the hexabromides. For most oils about 2 or 3cc. of the solution are required to produce a deep red color, which is considered indicative of a proper excess of bromine. After the addition of bromine, the weighing tube is allowed to stand in a refrigerator, temperature being 20°C., over night. Next morning it is cooled in ice water and centrifuged from two to four minutes, the solvent is then decanted from the precipitate, 10cc. of ice cold absolute ether added, the precipitate stirred up with the ether, cooled in ice water, again centrifuged two to four minutes and the ether decanted. This washing is repeated twice more and after decanting the final wash ether, the weighing tube is dried in an oven at 100°C. to constant weight, once half hour being sufficient. In the case of Salmon oils which gave a very large percentage of hexabromides, a weighed quantity of the oil was mixed with a weighed quantity of a cottonseed oil, which by test had shown no hexabromide precipitate, and the hexabromide value was then determined upon the mixed oil and calculated back to the original Salmon oil. This was found necessary as a very bulky hexabromide precipitate could not be readily centrifuged and washed rapidly enough to prevent the solution warming up and dissolving some of the hexabromides. In order to get concordant results with an empirical method of this kind, of course every precaution must be taken to work always under exactly the same conditions. After a little experience in the manipulation of this method, it is possible to obtain duplicate determinations which agree within 0.2 per cent with oils having a hexabromide value of 25 to 50.

The iodine number was determined upon a separate portion of each sample by the regular official Hanus method."

By a determination of the percentage of insoluble bromides in the oil, Bailey and Johnson have found it possible to identify the species of Salmon from which the various oils analysed have been prepared. By analogy between the formation of insoluble bromides from these oils and of products of similar physical

characteristics from vegetable drying oils, the name "hexabromides" has been applied to these products. In an effort to determine the correctness of this assumption the following experimental work has been carried out.

EXPERIMENTAL

Preparation of Fatty Acids

A 92.0020 gram sample of Salmon oil was weighed out. To this was added a solution of 20 grams potassium hydroxide in 50cc. of water followed by 50cc of 95% ethyl alcohol. This was placed on the steambath and shaken frequently until complete saponification took place, about two hours. The alcohol was then allowed to boil off, the soap dissolved in water, put in a separatory funnel and acidified with hydrochloric acid. After the separation of the free acids as an oily layer, the water was drawn off, the acids taken up in ether and washed repeatedly with distilled water until the washings showed no red color with methyl orange, indicating the absence of mineral acids. Then, the ether solution was allowed to dry over anhydrous sodium sulfate, filtered, and made up to 500cc. with dry ether.

Bromination

The method of Eibner and Muggenthaler[9] was used.

250cc. of the ether solution of acids, representing 46.004 grams of Salmon oil, were brominated with an excess of bromine, maintaining the temperature of the mixture at -5°C. by means of a bath of ice and salt. The bromine was added very slowly from a dropping funnel and the mixture shaken continuously until the red color of the mixture showed an excess of bromine present. After allowing the brominated mixture to stand overnight, the upper ether layer was decanted and the bromides washed five times with dry ether, using 500cc. for each washing. The bromides were allowed to remain in contact with each portion of wash ether for

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

at least twelve hours. Being very finely divided, the bromides require this length of time to settle completely. The mixed bromides form a pure white amorphous powder very difficult to handle. Finally the insoluble bromides were filtered in a three inch Buchner funnel and washed with dry ether, using suction each time. The bromides were allowed to dry in air, suction being maintained, overnight, and then the drying finished in a vacuum dessicator. Then they were ground to a very fine powder in a mortar, placed in a weighing tube, from which each sample was weighed by difference.

Bromine determination

Bromine was determined in the organic bromides by the Sodium Peroxide Fusion Method with a Parr Bomb following the Lemp and Broderson[10] modification, using hydrazine sulfate to reduce the silver bromate formed.

Proceedure:

About 0.20 gram of the bromide was weighed into a fusion cup, 1 gram of potassium nitrate, 0.30 gram of benzoic acid, and one level measure (10 grams) of sodium peroxide added. The bomb was then closed, the contents thoroly mixed and a strong Bunsen flame applied until there was a red ring around the bottom of the bomb - in 2-4 minutes. The bomb was then cooled under the tap, opened, and the contents cautiously decomposed in 200cc. of distilled water in a 600cc. beaker covered with a watch glass. 20 - 25 cc. of concentrated nitric acid were then added, until the ferric oxide in suspension was completely dissolved, yielding a clear solution. Then an excess of standard silver nitrate solution was added and the contents of the beaker boiled for half a minute, after which 20cc. of a four percent solution of hydrazine sulfate were added, and warming continued until bubbles of nitrogen gas ceased to be evolved. The contents of the beaker were then cooled, 5cc. of ferric alum indicator added, and the excess silver nitrate titrated with standard ammonium thiocyanate. The percent of bromine was then calculated.

PERCENTAGE OF BROMINE IN INSOLUBLE BROMIDES.

Wt. of Bromide	cc. Ag NO_3	N.F. Ag NO_3	cc. NH_4 CNS	N.F. NH_4 CNS	% Bromine
.3076	30cc.	.1141	8.40	.0848	70.45
.1439	30	"	25.35	"	70.54
.1621	30	"	23.35	"	70.99
.1178	20	.1252	13.50	.1025	70.56

CONCLUSIONS

The mean of the above analyses shows a bromine content for the ether insoluble brominated acids of Salmon oil of 70.63%. The bromine content of hexabrom stearic acid, obtained by saturating linolenic acid with bromine, 62.28%. That of octobromstearic acid, obtained from clupanodonic acid in the same way, is 68.92%. From this it would appear that linolenic acid is not present in Salmon oil, and that the term "hexabromides" applied to the insoluble brominated fat, is not correct and should not be used.

The exception to this statement may be found in the possible occurrence in such oils of acids more highly unsaturated than clupanodonic. The bromine derivatives of these, mixed with hexabromstearic acid in proper proportions, would have a bromine content equal to that which we have found on analysis. But even in such an instance there is no reason for the application of the term "hexabromides" to such a product.

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